FORM PTO-1390 (Modified) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE ATTORNEY'S DOCKET NUMBER
(REV 5-93)

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

Rec'd PCT/PTO 30 NOV 1998

065691/0145

U.S. APPLICATION NO (If known, see 37 C.F.R. 1.5)

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/FR97/00944	**May 30, 1997**	**May 31, 1996**

TITLE OF INVENTION

RECOMBINANT PLANT GENOME COMPRISING SPECIFIC CHICORY GENES AND A NUCLEOTIDE SEQUENCE CONFERRING MALE STERILITY, AND ITS USE

APPLICANT(S) FOR DO/EO/US

Louis DELESALLE, Charles DHELLEMMES, and Michel DESPREZ

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☐ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☒ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☒ A translation of the International Application into English (35 U.S.C. 371 (c)(2)).

7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.

8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.

14. ☐ A substitute specification.

15. ☐ A change of power of attorney and/or address letter.

16. ☐ Other items or information:

U.S. APPLICATION NO. (If known, see 37 C.F R 1 50	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
	PCT/FR97/00944	065691/0145

17. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5):		
Search Report has been prepared by the EPO or JPO **$840.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) **$670.00**		
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2) **$760.00**		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$970.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) **$96.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 840.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e))	$ 0.00	

Claims	Number Filed	Number Extra	Rate		
Total Claims	15 -20 =	0	X $18.00	$ 0.00	
Independent Claims	4 -3 =	1	X $78.00	$ 78.00	
Multiple dependent claim(s) (if applicable)			+ $260.00	$ 260.00	
TOTAL OF ABOVE CALCULATIONS =				$ 1,178.00	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				$ 0.00	
SUBTOTAL =				$ 1,178.00	
Processing fee of **$130.00** for furnishing English translation later the ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f). +				$ 0.00	
TOTAL NATIONAL FEE =				$ 1,178.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$ 40.00	
TOTAL FEES ENCLOSED =				$ 1,218.00	
				Amount to be: refunded	$
				charged	$

a. ☒ A check in the amount of $1,278.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. 19-0741 in the amount of $ to the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 19-0741. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Foley & Lardner
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Patricia D. Granados
SIGNATURE

Patricia D. Granados
NAME

33,683
REGISTRATION NUMBER

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No. 065691/0145

In re patent application of
Delesalle *et al.*
Serial No. Unassigned
Filed: November 30, 1998
For: RECOMBINANT PLANT GENOME COMPRISING SPECIFIC CHICORY GENES AND A NUCLEOTIDE SEQUENCE CONFERRING MALE STERILITY, AND ITS USE

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination of the above-identified application, Applicants respectfully requests that the following amendment be entered into the application:

IN THE CLAIMS:

Please amend Claim 6 as follows:

6. Cytoplasm according to claim 5, characterized in that it comprises mitochondria comprising at least one nucleotide fragment of 347 bp which is borne by the orf 522 sequence or a sequence with at least 50% homology with the said fragment [according to Claim 3].

Attorney Docket No.

REMARKS

Entry of the foregoing amendment prior to examination is respectfully requested.

Respectfully submitted,

November 30, 1998

Patricia D. Granados
Reg. No. 33,683

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RECOMBINANT PLANT GENOME COMPRISING SPECIFIC CHICORY GENES AND A NUCLEOTIDE SEQUENCE CONFERRING MALE STERILITY, AND ITS USE

The present invention relates to the use of nucleotide sequences which allows cytoplasmic-type male sterility to be imparted to plants of the genus Cichorium.

The genus Cichorium includes plants which are of great interest in the agrifood industry, such as the various types of chicory and endives.

These are plants which have male and female reproduction systems at the same time and are therefore capable of self-fertilization. Such an event is undesirable when it is desired to carry out crosses with a plant of another variety in order to obtain hybrids.

In Cichorium intybus, plants which exhibit nuclear male sterility have already been proposed. However, cytoplasmic male sterility is an interesting solution for the production of hybrid species.

Cytoplasmic male sterility is a characteristic that is transmitted by the female parent of a plant (maternal inheritance) and which prevents the formation of viable pollen. Good-quality male sterility must not affect female fertility of the plant to allow it to be crossed with male fertile plants. It is therefore desirable to have available a system which imparts such stable cytoplasmic male sterility.

It is also desirable to have available a reliable marker of this cytoplasmic male sterility which allows plant cells to be selected even before the development of a complete plant which exhibits all of the phenotypic characteristics.

The present invention therefore relates to a recombinant plant genome, characterized in that it comprises specific chicory genes and a nucleotide sequence conferring male sterility, which is borne by the sunflower orf 522 sequence or by a sequence with at

least 50%, advantageously at least 90%, homology with the said orf 522 sequence.

The orf 522 sequence is a sequence which was revealed in sunflower (Helianthus), in particular H. annuus, where it seems to be associated with cytoplasmic male sterility (Köhler et al, Mol. Gen. Genet., 1991, 227: 369-376).

The applicant has found that the presence of this orf 522 sequence in the genome of a plant of the genus Cichorium was linked to cytoplasmic male sterility.

Sequences which are suitable for carrying out the invention comprise sequences borne by the above-described orf 522 sequence, as well as by sequences with a similarity of at least 50%, preferably at least 80%. Suitable sequences advantageously show a similarity of 90% with the said orf 522 sequence, and comprise in particular the sequences which encode the same protein, taking into consideration the degeneration of the genetic code, or a protein in which certain amino acids were replaced by equivalent amino acids. The term "Equivalent amino acids" is understood to mean amino acids which have similar chemical behavior and/or similar molecular weights. They also comprise sequences which encode a protein in which one or more amino acids which are not essential for the activity have been deleted or replaced.

The genome can be of the nuclear or mitochondrial type.

When the sequence is present in the nuclear genome, the latter will also comprise a pre-sequence which allows the translation product of this sequence to enter the mitochondria.

The recombinant genome is preferably a mitochondrial genome. The invention therefore also relates to a mitochondrion which comprises a recombinant genome as defined above.

In particular, the invention relates to a mitochondrion, characterized in that it comprises at

least one nucleotide fragment of 347 bp which is borne by the orf 522 sequence or a sequence with at least 90% homology with the said fragment.

Within the orf 522 sequence (Köhler et al, Mol. Gen. Genet 1991), the 347 bp sequence is flanked by primers of the sequences:

SEQ ID NO 1: 5'CCCCCTCCCTGGTGGATCCGGCG3'
SEQ ID NO 2: 5'CCCTCTATGAGTACCGTTCTCTCACG3'

The invention relates to a recombinant plant cytoplasm, characterized in that it comprises a nucleus comprising the genome of the genus Cichorium and a recombinant genome defined hereinabove, in particular a cytoplasm which comprises mitochondria comprising a nucleotide sequence borne by the Helianthus annuus orf 522 sequence or by a sequence with at least 50% homology.

The recombinant plant cells comprising such a cytoplasm are within the scope of the invention, in particular a plant cell which comprises a nucleus comprising essentially the genome of a species selected from amongst Cichorium intybus and Cichorium endivia.

Without limiting the invention in any way, the following cultivation groups may be mentioned amongst these species:

Cichorium intybys L:
- "wild improved" chicories
- "Barbe de Capucin" chicories
- "sugar loaf" chicories
- "Chioggia" chicories
- "Verona" chicories
- "Catalonia" chicories
- "Treviso" chicories
- "Variegato di Castelfranco" chicories
- "Witloof" chicories (or "Brussels" chicory or "chicon" chicory)

- "Soncino" chicories

"Industrial" chicories (for roasting and for sugars)

- "fodder" or "game" chicories...

Cichorium endivia L.: - "Scarole" endives

- "frisée" endives ...

These are so-called large-rooted chicories (industrial, fodder and Witloof) or so-called salad chicories (green, red, variegated, for forcing or not).

The expert will be able to apply the invention without difficulty, in particular by referring to "Génétique et Amélioration de la Chicorée industrielle" [Genetics and Improvement of Industrial Chicory], DESPREZ et al., specialist meeting on November 30, 1994, at the Académie d'Agriculture de France, No. 80 (7) 48-49.

According to another aspect the invention relates to a method of producing a plant of the chicory genus or of the reproduction material of this plant which exhibits cytoplasmic male sterility, characterized in that a nucleotide sequence borne by the sunflower orf 522 sequence or by a sequence with at least 50%, preferably at least 90%, homology with the said orf 522 sequence, is integrated into the cell genome of this plant. The scope of the invention extends to the plant (or the production material), with the exception of plant varieties.

It also relates to an essentially non-biological method of preparing plant hybrids, characterized in that a plant which can be obtained by the above-described method is crossed with a plant of the same species which lacks the Helianthus annuus orf 522 sequence or a sequence which has at least 90% homology with the said orf 522 sequence.

In another aspect the invention relates to a method of selecting cytoplasmic male sterility in a plant of the genus Cichorium, characterized in that the mitochondrial nucleic acid of the plant is brought into

contact with a labeled probe comprising at least 10 nucleotides of the orf 522 sequence.

Other variants and characteristics of the invention will become clear by reading the examples which follow:

Example 1 : Preparation of the plant material

Seeds of Cichorium intybus L. cv. Pévèle were provided by Florimond Desprez, and (cytoplasmic male-sterility, or SMC) Helianthus annuus seeds were obtained commercially.

The Cichorium intybus seeds were surfaced-sterilized with a 0.1% (w/v) $HgCl_2$ solution, washed three times with distilled water and placed into Petri dishes on Heller's culture medium (1953)(macro- and microsalts, without $FeCl_3$) supplemented with 19.5 mg/l Fe-EDTA; 20 g/l sucrose and 6 g/l agar (Bioakar type E) and cultivated under the culture conditions described by Rambaud et al (1990). The aseptic seedlings were then transferred onto the same medium in culture tubes. The Helianthus annuus seeds were sterilized with a 50 g/l calcium hypochlorite solution and then washed three times with distilled water and transferred into a solution of sucrose (10 g/l)/agar (0.6%).

Chicory leaves were harvested from 12- to 14-day old plants. The leaves were cut into pieces and incubated in a solution with 15 g/l caylase 345, 0.5 g/l caylase M2 (Cayla, Toulouse, France) and 90 g/l mannitol.

As far as the sunflower seeds are concerned, the hypocotyls were removed 6 to 10 days after germination and incubated in the same maceration solution.

The protoplasts were incubated for 5 hours and 30 minutes at 30°C in the dark without moving, purified by filtration through 50 µm-mesh sieves, harvested and washed three times by low-speed centrifugation (100×g) for 15 min.

After the supernatant had been removed using a Pasteur pipette, the protoplasts were mixed in a ratio of 1:3 (sunflower/chicory) to obtain a suspension comprising from 7 to 11.10^6 protoplasts/ml.

The protoplasts were fused by the method of Kao (Wetter LRL and Constabel F (eds) Plant Tissue Culture Methods, ch 7, pp 49-56, 1982), with the following modifications: one volume of a solution of protoplast mixture was placed into a Petri dish, and three volumes of a solution of 30% polyethylene glycol (PEG 4000 Serva) with 10% dimethyl sulfoxide (DMSO) were added dropwise. After gentle homogenization, the solution was left to rest for three minutes. One minute later, another 3.5 volumes of Kao's solution number 3 (1982) are added. Then, 3.5 volumes of Kao's solution number 3 (1982) are added, and 3 minutes later 6 × 3.5 volumes of washing medium (Saksi N et al. CR Acad. Sci. Paris 302: 165-170, 1986) are added. The protoplasts are harvested by centrifugation (8 min, 100 × g) after 10-15 minutes; they are then washed 3 times with 8 ml aliquots of the washing medium and resuspended in MC1 culture medium (Saksi et al, 1986), in which the concentration of 1-naphthylacetic acid (NAA) is 2 mg/l, the inositol concentration is 250 mg/l and the KNO_3 concentration is 144 mg/l.

After one or two days of culture in this medium to a density of 2.10^4 protoplasts/ml, the heterokaryocytes isolated are cultured at low density (12/100 µl) at 30°C in a modified MC1 medium (0.5 mg/l NAA) to which are added 2-(N-morpholino)ethanesulfonic acid (MES) (5 mM), casein hydrolysate (150 mg/l) and coconut milk (2%; v/v).

One month later, the colonies obtained from the fusions of heteroplasmic protoplasts are transferred to a proliferation medium and then onto a regeneration medium (Rambaud et al. 1990). After rooting, the plants were transferred to the greenhouse for several weeks and then transplanted into the field.

The plants obtained have a chicory phenotype. Amongst them, the line designated CT 52/3 shows male sterility by lack of anther dehiscence; line CT 41/1 shows male sterility by the complete absence of anthers.

Example 2

Materials and methods

Plant material

Sixteen populations of industrial chicories with normal cytoplasm numbered FD1 to FD16, the industrial chicory cv. Pévèle, and a family of industrial chicory (CT 41/1) 20540 U with male cytoplasmic sterility (cms) provided by the company Florimond-Desprez, a chicory CT 52/3 (cms), the sunflower cv. Mirasol (cms). The young leaves were removed from greenhouse-grown plants and stored at -80°C.

DNA

Adaptation of the protocols of the Dellaporta-laboratory (Plant. Mol. Report., 1983) for total DNA miniextraction: the steps were carried out at 4°C. Finely grind with liquid nitrogen a leaf section of 150 to 200 mg fresh weight. Transfer into a 2 ml microtube. Add 940 µl of buffer solution Tris-HCl 0.1 M pH 8.0, EDTA 50 mM, NaCl 0.5 M, β-mercaptoethanol 10 mM. Add 62 µl of 20% SDS and vortex vigorously. Incubate for 15 minutes at 65°C. Add 310 µl of 5 M potassium acetate and vortex vigorously. Leave to precipitate for 30 minutes. Centrifuge. Centrifuge for 15 minutes at 17,500 g. Transfer 1 ml of the supernatant into a 2 ml microtube. Add 0.5 ml of isopropanol and mix. Leave to precipitate for 15 minutes. Centrifuge for 10 minutes at 12,000 g. Decant the supernatant using a micropipette. Dry the pellets in a Speed-Vac apparatus for 10 minutes at a low temperature setting. Redissolve the pellets in 100 µl of buffer solution Tris-HCl 50 mM

pH 8.0, EDTA 10 mM, ribonuclease A 0.2 mg/ml and incubate for 2 hours at 37°C. Extract three times with phenol and then once with chloroform. Add 0.1 volume of 3 M sodium acetate pH 5.2 and two volumes of absolute ethanol at 4°C. Leave to precipitate for 15 minutes at 4°C. Centrifuge for 10 minutes at 12,000 g. Wash the pellet with 70% ethanol at 4°C. Centrifuge for 3 minutes at 12,000 g. Decant using a micropipette and dry for 10 minutes in the Speed-Vac at low temperature. Take up the pellet in a buffer solution of Tris/HCl 1 mM ph 8.0, EDTA 0.1 mM.

Quantify a 5 µl aliquot by horizontal electrophoresis in 08% agarose gel, TBE ix, EtBr 0.5 µg/ml.

PCR

Two primers of 23 and 26 bases which flank a 347 bp fragment within the orf 522 sequence (Köhler et al., Mol. Gen. Genet., 1991).

Primer sequences:

SEQ ID NO 1: 5'CCCCCTCCCTGGTGGATCCGGCG3'

SEQ ID NO 2: 5'CCCTCTATGAGTACCGTTCTCTCACG3'

The 60 Bio-Med thermocycler is programed as follows: first cycle: 3 min, 92°C; 30 cycles: 1.30s, 92°C; 2.30s, 55°C; 3.1 min, 72°C; last cycle: 5 min, 72°C. Reaction mixture: Appligène buffer 1x: Tris-HCl 10 mM pH 9.0. Triton X-100 0.1%, $MgCl_2$ 1.5mM, BSA 0.2 mg/ml; dNTP 100 µM; primers 0.2 µM each; Appligène Taq polymerase 2 U/100 µl; DNA matrix 50 ng/100 µl; H_2O to 10 µl; mineral oil: 50 µl. Analysis of the products by horizontal electrophoresis in 1.6% agarose gel, TBE 1x, EtBr 0.5 µg/ml.

Hybridizations

Traditional transfer technique by the Southern method ("Maniatis"). Chemical labeling of the orf 522 probe using the Dig-High-Prime kit (Böehringer-Mannheim). Visualization as described in the Böehringer-Mannheim protocol using CSPD (Tropix) as

chemoluminescent substrate. Exposure time of the membranes: from one hour to one night in the case of very weakly amplified fragments.

Results

The total DNA of 4 individuals per population FD1 to FD16, 8 individuals of the family 20540 U, of chicory CT 52/3 and of sunflower "Mirasol" were extracted, that is a total of 73 individuals. Absence of inhibition of the PCR by impurities present in the DNA extracts was checked by adding several tens of femtograms of the 347 bp fragment to each reaction mixture. All the PCR analyses of the 52/3 DNA, sunflower "Mirasol" DNA and the DNA of the family 20540 U allow amplification of a substantial quantity (approx. 200 ng) of the approx. 350 bp fragment of orf 522. All the chicories with a normal cytoplasm lack this fragment. Analysis of these PCR products by molecular hybridization with the aid of an orf 522 probe prepared by PCR from total DNA of Mirasol sunflower confirms the homology between the amplified fragment in the (cms) chicories and the probe.

Conclusion

The results obtained have demonstrated that orf 522 is not present in fertile chicories. The determination by means of PCR of the presence/absence of the orf 522 sequence can therefore be considered for routine analysis.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: FLORIMOND DESPREZ VEUVE ET FILS
(B) STREET: BP 41
(C) CITY: CARPELLE EN PEVELE
(E) COUNTRY: PARIS
(F) POSTAL CODE: 59242

(ii) TITLE OF THE INVENTION: RECOMBINANT PLANT GENOME, MITOCHONDRION AND CELL COMPRISING IT, AND METHOD OF SELECTING CYTOPLASMIC MALE STERILITY IN A PLANT OF THE GENUS CICHORIUM

(iii) NUMBER OF SEQUENCES: 2

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(vi) DATA OF THE EARLIER APPLICATION:
(A) APPLICATION NUMBER: FR 9606725
(B) FILING DATE: 31 MAY 1996

(2) INFORMATION FOR SEQ ID NO: 1

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

CCCCCTCCCT GGTGGATCCG GCG 23

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

CCCTCTATGA GTACCGTTCT CTCACG 26

PATENT CLAIMS

1. Recombinant plant genome, characterized in that it comprises specific chicory genes and a nucleotide sequence conferring male sterility, which is borne by the sunflower orf 522 sequence (Helianthus annuus) or by a sequence with at least 50% homology with the said orf 522 sequence.

2. Mitochondrion, characterized in that it comprises a recombinant genome according to Claim 1.

3. Mitochondrion according to Claim 2, characterized in that it comprises at least one nucleotide fragment of 347 bp which is borne by the orf 522 sequence or a sequence with at least 50% homology with the said fragment.

4. Recombinant plant cytoplasm, characterized in that it comprises a nucleus comprising the genome of the genus Cichorium, and a recombinant genome according to Claim 1.

5. Cytoplasm according to Claim 4, characterized in that it comprises mitochondria comprising a nucleotide sequence borne by the Helianthus annuus orf 522 sequence or by a sequence with at least 50% homology.

6. Cytoplasm according to Claim 5, characterized in that it comprises mitochondria according to Claim 3.

7. Recombinant plant cell, characterized in that it comprises a cytoplasm according to one of Claims 4 to 6.

8. Plant cell according to Claim 7, characterized in that it comprises a nucleus comprising essentially the genome of a species selected from amongst Cichorium intybus and Cichorium endivia.

9. Method of producing a plant of the chicory genus or the reproduction material of this plant which exhibits cytoplasmic male sterility, characterized in that a nucleotide sequence borne by

the Helianthus annuus orf 522 sequence or by a sequence with at least 50% homology with this orf 522 sequence is integrated into the cellular genome of the said plant.

10. Use of the Helianthus annuus orf 522 sequence or of a sequence with at least 90% homology wih the orf 522 sequence to confer cytoplasmic male sterility to a plant of the genus Cichorium.

11. Method of selecting cytoplasmic male sterility in a plant of the genus Cichorium, characterized in that the mitochondrial nucleic acid of the plant is brought into contact with a labeled probe comprising at least 10 nucleotides of the orf 522 sequence.

Docket No. _____

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Recombinant plant genome comprising specific chicory genes and a nucleotide sequence conferring male sterility, and its use

the specification of which is attached hereto unless the following box is checked:

☒ was filed on May 30, 1997 as United States Application Number or PCT International Application Number PCT/FR97/00944 and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is known by me to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed:

PRIOR FOREIGN APPLICATION(S)

NUMBER	COUNTRY	DAY/MONTH/YEAR FILED	PRIORITY CLAIMED
96 06725	France	31.05.1996	Yes

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

APPLICATION NO.	FILING DATE

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is known by me to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

APPLICATION SERIAL NO.	FILING DATE	STATUS: PATENTED, PENDING, ABANDONED

I hereby appoint as my attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: Stephen A. Bent, Reg. No. 29,768; David A. Blumenthal, Reg. No. 26,257; William T. Ellis, Reg. No. 26,874; John J. Feldhaus, Reg. No. 28,822; Patricia D. Granados, Reg. No. 33,683; John P. Isacson, Reg. No. 33,715; Donald D. Jeffery, Reg. No. 19,980; Eugene M. Lee, Reg. No. 32,039; Richard Linn, Reg. No. 25,144; Peter G. Mack, Reg. No. 26,001; Brian J. McNamara, Reg. No. 32,789; Sybil Meloy, Reg. No. 22,749; George E. Quillin, Reg. No. 32,792; Colin G. Sandercock, Reg. No. 31,298; Bernhard D. Saxe, Reg. No. 28,665; Charles F. Schill, Reg. No. 27,590; Richard L. Schwaab, Reg. No. 25,479; Arthur Schwartz, Reg. No. 22,115; Harold C. Wegner, Reg. No. 25,258.

Address all correspondence to FOLEY & LARDNER, Washington Harbour, 3000 K Street, N.W., Suite 500, P.O. Box 25696, Washington, D.C. 20007-8696. Address telephone communications to _____ at (202) 672-5300.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

100

Full Name of First or Sole Inventor	Signature of First or Sole Inventor	Date
DELESALLE Louis		27/11/98
Residence Address		Country of Citizenship
1 bis rue de Thouars, 59242 Cappelle en Pevelle, France		France
Post Office Address		
The same as residence		

200

Full Name of Second Inventor	Signature of Second Inventor	Date
DHELLEMMES Charles		27/11/98
Residence Address		Country of Citizenship
33 rue Ladrerie, 59242 Cappelle en Pevelle, France		France
Post Office Address		
The same as residence		

300

Full Name of Third Inventor	Signature of Third Inventor	Date
DESPREZ Michel		27/11/98
Residence Address		Country of Citizenship
Les Diablotins, rue Florimond Desprez, 59242 Cappelle en Pevelle, France		France
Post Office Address		
The same as residence		

Full Name of Fourth Inventor	Signature of Fourth Inventor	Date
Residence Address		Country of Citizenship
Post Office Address		

Full Name of Fifth Inventor	Signature of Fifth Inventor	Date
Residence Address		Country of Citizenship
Post Office Address		